RESISTANCE UNDER HIGH TEMPERATURE TO ROOT-KNOT NEMATODES (*Meloidogyne* spp.) IN TOMATO (*Lycopersicon* spp.)

By

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Abstract of Dissertation Presented to the Graduate Council
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Experiments were conducted to determine why the Mi gene for resistance to root-knot nematodes was not effective in Florida and to identify new sources of resistance in tomato. In greenhouse experiments conducted in controlled temperature tanks the best inoculum level for screening tomato accessions at high temperature (32.5 C) for resistance to Meloidogyne incognita and M. javanica was 200 eggs per plant. At this inoculum level the frequency of individual egg masses was low enough to be counted, making the measurement of quantitative resistance feasible.

Race 4 of M. incognita was found to be more aggressive toward tomato accessions than race 1. A few selections from Lycopersicon peruvianum var. dentatum PI 129149 showed more resistance to race 4 than L. esculentum lines. High resistance to race 1 was found in both Lycopersicon species tested at high temperature. The best selection for overall resistance was PI 129149-11-5-16, which was resistant to M. incognita at high temperature and produced many progeny immune to

race 1 of M. incognita at initial inoculum density of 200 eggs per plant.

The critical testing period for studies of resistance to M. incognita at high temperature appears to be the first 12 days after inoculation with nematode eggs. The period from 12 to 30 days after inoculation had little effect on nematode reproduction. Thermoperiodism was found to play an important role in maintaining the resistance to M. incognita in resistant tomato accessions. Under diurnal soil temperature changes of 32.5 C (day) and 20-25 C (night) the plants were able to decrease significantly the nematode reproduction, as compared to continuous day/night high temperature. In field experiments several cultivars and F_1 hybrids carrying the Mi gene were inoculated with 10,000 eggs of M. incognita, race 4. This treatment seriously reduced yield, the number of fruits, and the average fruit weight.

INTRODUCTION

The group of root-knot nematodes (Meloidogyne spp.) is one of the most important among plant-parasitic nematodes (76), and probably causes more crop damage than any other nematode group (9). The importance of this group was recognized by the Agency for International Development in creating the International Meloidogyne Project which works with more than 70 cooperators around the world (76).

Florida is a major tomato (Lycopersicon esculentum) producing state in the USA with an area harvested of 16,807 hectares and a total production of 408,827 tons in 1977/78 (35). However, "Florida, by virtue of its rather higher ambient temperature, high relative humidities, and comparatively high annual rainfall, provides an environment in which tomato diseases flourish" (20, p. i). The tomato breeding program in Florida is working toward multiple-disease resistance and has incorporated several important disease resistance genes into many cultivars during the past 55 years. Although many problems have been solved by the release of resistant cultivars, others, including root-knot nematodes, still challenge the tomato breeder in Florida. Walter et al. (87) reported in 1949 that some lines showed a moderate type of root-knot nematode resistance. They also tested 6 accessions from the Hawaii Agricultural Experimental Station and found that they could not be classified as free of root knot. A few years later it was decided to cease working with root-knot nematode resistance, and in 1967 Walter (86) suggested

that the high temperature may be the main reason that the resistance due to the Mi gene was never highly effective in Florida.

The main Florida cultivars Walter (15) and Floradade (81) do not have resistance to root-knot nematodes. A program to search for the source of resistance which does not break down at high temperature was initiated at the Agricultural Research and Education Center (AREC), Bradenton, Florida. The present work is a part of this program and includes the screening of several Lycopersicon accessions using controlled temperature tanks. The screening experiments studied the influence of inoculum densities, thermoperiod, length of initial exposure time to different temperatures, and nematode races on the manifestation of resistance to Meloidogyne spp.

LITERATURE REVIEW

The Root-Knot Nematodes

Historical

In 1949, Chitwood (16) revised the genus Meloidogyne Goeldi, 1887. Before that revision all species of Meloidogyne were included in the single species Heterodera marioni. Chitwood revived the genus Meloidogyne and described 4 species: M. javanica, M. incognita, M. hapla, and M. arenaria, which were found later to be the most widespread in the world. He also described a subspecies of M. incognita and classified it as M. incognita var. acrita, which in 1976 Esser et al. (32) reclassified to the rank of a species, M. acrita. As knowledge advanced in the new discipline of nematology and the number of nematologists increased new species were described and rigorous criteria for identifying races were developed (66, 74, 76). By the end of 1976, 36 species of Meloidogyne had been named (76).

Biological Races

According to Webster (89), "regardless of the morphology of a nematode, its ability to parasitize a certain host depends on the physiological and ecological aspects of the host-parasite relationship, which is controlled by the genetical constitution of the host and the parasite. In several species of plant nematodes physiological

and ecological data have resulted in species being subdivided into biological races, pathotypes or strains" (p. 27).

Differential hosts are now used in separating the races of Meloidogyne spp. based on host reaction (73, 74, 76). Southards and Priest (73), using host differential tests, were able to distinguish 6 physiological races among 17 populations of M. incognita in Tennessee. In population samples from many parts of the world only 4 races of M. incognita have been designated according to their parasitism on NC 95 tobacco and Deltapine 16 cotton (65, 66, 76). Taylor and Sasser (76) also recognized two widespread races of M. arenaria, one race of M. javanica, and one race of M. hapla.

The existence of races should be kept in mind for a possible explanation of discrepancies in research results and the failure of resistant genotypes to perform as expected in the fields (65). Riggs and Winstead (61) found that larvae of M. incognita, M. acrita, and M. arenaria when occasionally matured on roots of the resistant Hawaii 5229 tomato were able to develop mutant races which were highly aggressive with respect to Hawaii 5229. They stated that the appearance and establishment of mutants with different selective pathogenicity may complicate breeding programs for disease resistance. Taylor and Sasser (76) suggested that a resistance breaking race of a Meloidogyne species can become dominant in a field only when a resistant cultivar is grown in monoculture for several years. If it is grown in rotation with other crops, it can be cultivated in the same field without significant loss of resistance.

Life Cycle

The one-celled stage eggs of Meloidogyne spp. are normally deposited in a gelatinous matrix called an egg mass. After several mitotic divisions an egg develops into a first-stage larva. After the first molt the larva inside the egg becomes a second stage larva, and shortly afterward the larva hatches (76). In Florida, Meloidogyne arenaria overwinters as a second stage larva, and it is apparent that this stage constitutes a survival stage (37). The second larval stage is the infective one.

Root-knot nematode larvae penetrate directly through the root epidermis at or near the root tip and migrate intercellularly in the cortex (10, 17, 31). They orient their heads toward the stem and become established as sedentary parasites. They usually feed in the central vascular cylinder or stele (10, 17). Giant cells are formed (26) near the nematode head. Barrons (6) demonstrated that production of giant cells is necessary for the development of a root-knot nematode. Bird (11) reported that the development and maintenance of giant cells appeared to depend on a continuous stimulus from the nematode. The giant cells degenerate if the nematode is removed or destroyed. Giant cell formation in tomato (10) starts after the fourth day of penetration. The typical swelling of the root or gall (11) results from the enlargement of the nematode and from hypertrophy and hyperplasia in cortical and vascular parenchyma cells, pericycle cells, and endodermal cells surrounding the giant cells.

After penetration the larva undergoes three more molts with corresponding morphological and physiological changes. After the fourth

molt a larva becomes an adult. The male leaves the roots; males may or may not be necessary for reproduction. Finally, the female deposits one-celled stage eggs in an egg mass, and the entire cycle starts again (18, 76).

Normally most larvae develop into females (23, 76). The sex ratio varies according to the nutritional environment of the host and other factors that will be presented later. When the food supply is scarce, a large number of larvae may become males (23, 76).

Effects of Temperature on Root-Knot Nematodes

Most species of Meloidogyne are important plant parasites in tropical, subtropical, and temperate zones. In the United States, M. incognita, M. javanica, M. arenaria, M. acrita, and M. hapla are widespread, although the distribution varies with the species (64). South of Washington, D.C., M. incognita and M. acrita are the most common. North of this location M. hapla is the most common. M. javanica has been collected from scattered locations in the southern states. M. hapla and M. arenaria are fairly common where peanuts are grown, notably Alabama, Georgia, and Virginia (75).

Environmental factors influence the successful dissemination, establishment, and economic importance of a root-knot nematode. Among them temperature is of great importance. Temperature requirements for the establishment of M. incognita (on a probability basis) throughout the world have received (64) a high value of 7 out of 10 (10 = highly favorable).

Temperature may have its effects on nematodes in two ways (41): in its limitations on the survival and in the number of generations

that can be produced throughout the year. Several studies reported that different species of Meloidogyne have different temperature requirements for survival and reproduction (7, 14, 21, 25, 47, 78) and that each Meloidogyne has its optimum temperature range for development in each life stage (78, 80). Temperature also has a marked influence on the duration of a life cycle (22, 23, 60, 78), the rate of reproduction (78), and sex differentiation (23). According to Tyler (80) the minimum time required to complete the life cycle of a Meloidogyne spp. from larva to larva in tomato was 25 days at 27 C and 87 days at 16.5 C. Pyrowalakis (60) reached similar conclusions and also observed that five host plants, each one from a different botanical family--tomato, cucumber, endive, okra, and banana--had little effect on the time required for the completion of one life cycle in M. javanica. Tyler (80) reported that 6,500 to 8,000 heat-units (a heat-unit is a degree-hour above 10 C) was required for completion of one life cycle. The concept that the development of females and eggs of Meloidogyne is related to heat accumulation above a basal threshold temperature was also presented by Ferris et al. (34) and Milne and Duplessis (56). Temperature unfavorable for the development of the root-knot nematode may exercise a masculinizing effect on the developing larva (23). Males of M. incognita constituted less than 1% of the nematodes developed on Rutgers tomato at 20, 25, 30, and 35 C but 6.7% at 15 C.

Temperature affects the rate of reproduction, development, and hatch of eggs (12, 34, 83). M. javanica, M. acrita, and M. hapla reproduced on Rutgers tomato at 20, 25, and 30 C, but at 35 C M. hapla reproduction was extremely inconsistent. Two populations of M. acrita

and one of M. javanica were markedly reduced at 35 C. A population of M. javanica from black-eye bean produced numerous galls and egg masses at 35 C. At 20 C the reproduction of this population was slightly depressed (77). Daulton and Nusbaum (21) subjected M. javanica samples from Southern Rhodesia, North Carolina, and Georgia, and M. hapla from North Carolina to temperatures of 2 and 33 C. Eggs of M. hapla were more tolerant to 2 C and less to 33 C than those of M. javanica. The eggs of M. javanica from Southern Rhodesia were unaffected by 10 days exposure to 33 C, but the viability of eggs from Georgia and North Carolina populations was reduced markedly. It appears that each population has been influenced by the range of temperature that characterizes its natural environment.

Thomason and Lear (78) studied the influence of soil temperature on egg mass production by 6 populations of M. javanica and M. acrita, 2 of M. hapla, one of M. arenaria, and one of M. thamesi. Maximum egg production for most species was found to be in the range of 25-32 C. At temperatures above 30 C the number of egg masses produced by M. hapla was less than that produced by M. acrita and M. javanica.

Bird (13) reported that embryogenesis and hatching of eggs of M. javanica were suppressed by a brief heat treatment, 46 C for 10 minutes. The heat effect was greater on hatching than on embryogenesis.

Bergeson (7) related that at 26.6 and 32.2 C hatching of eggs of M. acrita reached a peak during the 4th through the 7th day of exposure. At 15.6 C the hatching rate remained nearly constant. For M. acrita he concluded that 10 C most closely approached the optimum temperature for survival of eggs or larvae. At this temperature the

eggs survived for more than one year. As temperature increased, the activity of larvae and the utilization of food reserves increased; and, consequently, their life span in the absence of a host decreased.

Effect of Inoculum Densities and Host Age

Initial inoculum levels and plant age should be considered in relation to plant injury by a specific nematode. Bergeson (8) demonstrated that the sensitivity of susceptible cultivars to root-knot nematode was correlated with seedling age and inoculum density. He inoculated Tecumseh tomato seedlings in the dicotyledon, 3 to 4-petiole, and 6 to 7-petiole stages with 0, 10, 100, 1,000, 10,000, and 50,000 larvae of M. incognita. After 6 weeks the plants were evaluated. Most of the seedlings inoculated initially in the dicotyledon stage were killed by 10,000 and 50,000 larvae and were severely stunted at the 1,000 larvae density. Plants inoculated in the 3 to 4-petiole or 6 to 7-petiole stage had progressively less growth as the inoculum density increased and as the age of the seedling at the time of inoculation decreased.

Wallace (82) reported that both plant age and inoculum density affected root-knot nematode reproduction. He inoculated Tatura Dwarf tomato seedlings at the 1, 2, and 3-week stage with 0, 500, 5,000, and 50,000 larvae of M. javanica. After 7 weeks he assessed the reproduction rate. Reproduction on two-week old plants was more than twice that on one-week old plants, and in both ages reproduction decreased with the increase in population density. The three-week old plants showed the least reproduction of all, and this was attributed to the

small size of the pots, which inhibited plant growth. He confirmed the observation that nematode reproduction increases as plant size increases and that older plants can tolerate nematode infection better than younger ones.

High inoculum levels also reduced the rate of nematode development and significantly increased the percentage of males of M. incognita and M. javanica on Rutgers tomato (23). The tendency toward maleness is due to food scarcity or other stress factors.

The Resistance to Root-Knot Nematodes in Tomato

Historical

In 1939, Bailey (3) developed a greenhouse test for screening tomato seedlings for root-knot nematode resistance. The method consisted of placing 3 grams of chopped galled roots in 5-cm pots, filling the pots with soil, and sowing 30 to 40 seeds per pot. The seedling roots were observed for induced galls 19 to 21 days after sowing. He tested thousands of tomato seedlings of five Lycopersicon species: L. esculentum, L. glandulosum, L. hirsutum, L. pimpinellifolium, and L. peruvianum. All the species except L. peruvianum were found to be susceptible. Of the original 299 L. peruvianum selections, 20 were found which were free of galls after growing in nematode infested soil for 6 months. He stated that "the ability of nematodes to become adapted to a new host species justifies a conservative attitude toward the ultimate selection of highly root-knot tolerant plants, but the recently conducted tests indicate that there are possibilities for the breeding of tolerant varieties" (p. 574).

In 1940 and 1941, Romshe (63) used field test trials to screen 550 lots of seeds, among which 390 lots were accessions of the Plant Introduction Division (PI) of the United States Department of Agriculture. The other lots were from tomato breeders and seed companies. Nine accessions of L. peruvianum were entirely free of root-knots. He suggested the possibility of using L. peruvianum as breeding stock to develop commercial cultivars.

In 1942 and 1943, Ellis (30) studied the resistance to root-knot nematode of the L. peruvianum PI 129928, PI 126298, and PI 126944. He concluded that these L. peruvianum accessions were highly resistant, but not immune, to root-knot nematode.

Lesley (51) tried in 1935 to obtain crosses between L. esculentum and L. peruvianum. Although no viable seeds were produced, it was reported that pollination with L. peruvianum stimulated fruit development. In 1944, Smith (72) reported that he used the embryo culture method described by Skirm (71) to obtain a culture of L. esculentum cv. Michigan State Forcing x L. peruvianum PI 128657. He cultured a few embryos beyond the heart-shaped stage, which occurred in fruits 30 to 40 days after crossing, and got normal plants from these embryos. He reported that the embryo culture procedure could be used successfully to secure hybrid seed of the species cross. Cuttings of this hybrid were distributed to several tomato breeders in Australia, Hawaii, and 8 states of mainland USA (86). In Florida, Walter (86) spent some time propagating it by cuttings.

Watts (88), using Smith's hybrid cuttings, succeeded in outcrossing the F_1 to L. esculentum for the first time. Watts conducted progeny tests for nematode resistance in raised beds in a greenhouse

where the mean temperature was kept above 32.2 C. He selected lines highly resistant to root-knot nematode. The fruit of these lines varied in diameter from 2.5 to 5.0 centimeters, and they varied in color from orange to bright red. These selections were the source of resistance for the successful nematode resistant lines and cultivars developed subsequently at the Hawaii Agricultural Experiment Station (36, 38, 40) and at the Texas Agricultural Experiment Station (42).

In 1957, Thomason and Smith (79) stated that "so far as known, all resistant tomato stocks now in use derive their resistance from a single cross, Lycopersicon esculentum (Michigan State Forcing) x L. peruvianum (PI 128657) made in 1943" (p. 180).

Inheritance

The genetics of resistance to root-knot nematodes has been studied by several tomato breeders. Watts (88) suggested that resistance was conditioned by two complementary dominant factors. By using pollen of Smith's hybrid on lines of L. esculentum he obtained three outcross seedlings, 1 moderately resistant and 2 very strongly resistant. One of these two was self-fertile and its progeny were tested for nematode resistance. Out of 588 seedlings 322 were free of galls and 266 were susceptible, giving a 9:7 ratio. McFarlane et al. (54) suggested that resistance was controlled by a small number of factors. Gilbert and McGuire (38) presented an F_2 segregation ratio of 13:3, which suggested 1 or 2 dominant genes; but later the same authors (39), using advanced breeding lines with homozygous resistance, obtained results which indicated that resistance was conditioned by one major gene.

The symbol Mi, from M. incognita, was given to this gene. It was reported that the transfer of the Mi gene to horticulturally acceptable lines was becoming easier as a result of the successive breaking of undesirable linkages in resistant plants.

The monofactorial character was confirmed by Barham and Winstead (4), Thomason and Smith (79), Winstead and Barham (91), and Ohekar (58). It was reported that the same gene conferred resistance not only to M. incognita, but also to M. acrita, M. javanica, and M. arenaria (4, 79, 91). The Mi gene did not confer resistance to M. hapla. Ohekar (58) reported that this gene did not confer resistance to M. arenaria. Barham and Winstead (4) demonstrated that in Hawaiian and Texan tomato lines with root-knot resistance the Mi gene was incompletely dominant.

Harrison (42) pointed out that the monofactorial resistance of a line may be increased by the addition of modifiers from the susceptible parent, but additional data are necessary before this can be established.

Fatunla and Salu (33) studied the inheritance of root-knot resistance with the cultivars Nematex, Rossol, and the susceptible line Ife 1, using an inoculum with 50 larvae per seedling of M. incognita. The F_2 from Nematex x Ife 1 and Rossol x Ife 1 fitted the 3:1 ratio. The F_2 from Rossol x Nematex gave a 15:1 ratio, suggesting independent assortment of two genes with epistatic interactions. A possible explanation is that Hawaiian lines were the source of the Mi gene in Rossol, and Texan lines were the source for Nematex.

Sidhu and Webster (69) identified three genes, 2 dominant and 1 recessive, for resistance to M. incognita. They were named $LMiR_1$, $LMiR_2$, and $LMir_3$ and are possessed by the cultivars Nematex, Small Fry,

and Cold Set, respectively. Cold Set was crossed to IPB, a susceptible cultivar, and the F_2 segregation ratio was 1:3. Nematex and Small Fry were crossed to susceptible cultivars and gave the ratio 3:1. The greenhouse temperature was 24 ± 3 C. It should be pointed out that Small Fry is an F_1 hybrid, but it was selfed to obtain homozygosity for resistance to M. incognita before the F_1, F_2, and backcross progenies were developed (69, 70). Subsequently, they studied the relationship of $LMiR_1$ to the Mi gene, possessed by Nematex and Anahu, respectively. They reported that the $LMiR_1$ and Mi genes were either identical or allelic, based on the absence of any segregation in the F_2 and testcross progenies. The relationship between the $LMiR_1$ and $LMiR_2$ genes was determined by crossing Nematex with Small Fry-1 and by crossing the F_1 with the cultivar Bonny Best. They determined that the 2 genes were closely linked with only 5.65 map units between them. No conclusive results were obtained concerning the relationship of the recessive gene $Lmir_3$ (Cold Set-1) with the $LMiR_1$ and $LMiR_2$ genes. These inconclusive results were probably due to cytoplasmic factors contributed by the cultivar Cold Set-1.

Mechanisms of Resistance

Resistance to root-knot nematode in tomato has been attributed to a hypersensitivity reaction (HR) (27, 28, 29) following penetration of second-stage larvae. The larvae of Meloidogyne enter roots of resistant plants as well as susceptible ones (5, 53, 59). The hypersensitivity reaction (HR) is a commonly occurring response in plants attacked by fungi, viruses, and bacteria (48, 49) which consists of a rapid death

of cells around the causal agent. The root-knot nematodes cannot feed or reproduce in the necrotic area and die. The plant injury is confined to a few cells. Hung and Rohde (45) made the best study on the subject in tomato. They inoculated seedlings of the tomato cultivars Nemared, Hawaii 7153, and B-5 with M. acrita at 20 C. Giant cells were formed, larvae developed to the adult stage, and histochemical tests indicated little or no accumulation of phenolic compounds in the susceptible cultivar B-5. Chlorogenic acid was identified as the major phenolic compound in healthy tomato roots. Roots of Nemared became locally discolored and necrotic when invaded by the root-knot nematode larvae, and a large amount of chlorogenic acid accumulated in the injured tissues. The larvae died within the necrotic areas, and giant cells did not develop. Hawaii 7153, considered to be moderately resistant, produced both necrotic lesions and galls with a few egg masses. Uninfected roots of Nemared, Hawaii 7153, and B-5 contained 0.58, 0.44, and 0.38 mg, respectively, of chlorogenic acid per gram of root-tissue. Roots infected by M. acrita contained more chlorogenic acid than did healthy roots of the same cultivar.

Other mechanisms of resistance have been proposed. Sawhney and Webster (67) applied metabolic inhibitors (cycloheximide, puromycin, and actinomycin D) to Bonny Best and Nematex which were infected with M. incognita. At high concentrations the inhibitors prevented the browning (HR) of cells surrounding the nematode and also prevented gall formation. They concluded that the hypersensitivity reaction is not the only mechanism of resistance of tomato plants to M. incognita and that some mechanisms of resistance other than HR may be operative within resistant plants.

Zacheo et al. (92) studied the mechanism of resistance of tomato cultivars to M. incognita. They used 12 cultivars, 4 resistant and 8 susceptible to the root-knot nematode, and observed that in susceptible cultivars the number of females in the roots were 27 times more (on the average) than in the resistant ones after 50 days at 25-27 C. They also concluded that the protein-hydroxyproline content (as measured by hydroxyproline content after hydrolyses of protein without free amino-acids) in the cell wall of non-inoculated roots showed little variation in either susceptible or resistant plants. The hydroxyproline content did increase with nematode infection, and this increase was higher in resistant cultivars. The protein-hydroxyproline content of the mitochondria decreased in infested roots of susceptible cultivars and increased in resistant ones. They concluded that the difference between nematode resistant and susceptible cultivars is the capability of the resistant ones to develop cyanide-insensitive respiration following nematode attack to counteract the effect of the root-knot nematode. Because ascorbic acid content was a factor controlling the development of cyanide-insensitive respiration (1), Arrigoni et al. (2) recently studied the relationship between ascorbic acid content and resistance in tomato plants to M. incognita. They used 2 susceptible cultivars and the 4 resistant cultivars, Rossol, VFN 8, Piersol, and Brech. The resistant ones were irrigated daily with lycorine, an inhibitor of ascorbic acid synthesis, and the susceptible ones irrigated with ascorbic acid. The reaction of these plants to attack by M. incognita was tested. A decrease in ascorbic acid produced by application of lycorine reduced resistance to the root-knot nematode. The artificial increase in ascorbic acid transformed susceptible plants into resistant

ones. The concentration of ascorbic acid in susceptible plants was not altered by nematode attack, but in all 4 resistant cultivars its concentration was increased. They suggested that ascorbic acid is mostly utilized in the synthesis of mitochondrial hydroxyproline protein (probably through proline hydroxylation), which controls the cyanide-resistant pathway.

Effect of Soil Temperature

Holtzman (44) observed that Hawaiian cultivars and selections with resistance to root-knot nematodes were more heavily galled when grown in nematode-infested soil at 30 and 34.5 C soil temperatures than when grown at lower temperatures. He obtained 20, 25, 30, and 34.5 C constant soil temperatures by growing potted plants in tanks filled with water while holding the water temperature constant. He suggested that constant soil temperature played a role in modifying the resistance of tomato to M. incognita and that the control of soil temperature would be necessary when screening tomatoes for resistance to M. incognita. Holtzman (43) stated that when tomatoes are galled during the summer, it may not be due to the appearance of a more virulent strain of the nematode, but to a reversible, high-temperature breakdown of the cultivar resistance. If this is true, the resistance would be restored when the temperature returns to normal.

Laterrot and Pecaut (50) grew the cultivars Anahu and Saint Pierre (susceptible) under 2 greenhouse temperatures--18/27 C (day/night) and 37/39 C (day/night). They observed no galls on Anahu at 18/27 C, but numerous galls and egg masses at 37/39 C. They concluded that the egg

masses laid on Anahu did not belong to a more aggressive race of root-knot nematode, but that nematode growth and reproduction resulted from the effect of high temperature on the resistance of Anahu to M. incognita.

Dropkin (27) studied the seedling resistance of Nematex to M. acrita grown in controlled temperature tanks. Resistance of one-day old seedlings inoculated and grown for 4 additional days fell progressively as the temperature was increased from 28 to 33 C. At 32 C, 76% of larvae grew in Nematex compared with 75% in the susceptible cultivar Enterpriser. Temperatures of 30 and 32 C also reduced the resistance of Nematex to M. thamesi and M. javanica.

Elevated temperature early in the infection period determined the course of parasite development (27). Nematex plants inoculated and maintained at 32 C for 3 days, and subsequently held at 27 C for 1 month, contained abundant galls and eggs. In reciprocal experiments, seedlings were inoculated and maintained at 27 C for 1 and 2 days and then were transferred to 32 C for a month. Numerous galls and eggs developed at 32 C after exposure to larvae for 1 day at 27 C, but very few nematodes were able to induce galls or develop to maturity at the higher temperature after prior incubation for 2 days at 27 C. It is important to notice that the seedling roots were washed after the initial incubation to remove second stage larvae that had not penetrated the roots yet. The washed seedlings were replanted in fresh soil and then moved to the appropriate temperature tank.

MATERIAL AND METHODS

Greenhouse Experiments

Greenhouse experiments were conducted in Gainesville, Florida, in a fiberglass greenhouse provided with a gas heating and a water pad cooling system. The light transmitted to the plants inside the greenhouse was about 75% of the light measured outside the greenhouse. The following equipment and procedures applied to all greenhouse experiments except where indicated. Soil temperature was controlled by using controlled temperature tanks equipped with heating and cooling systems (capable of holding temperatures to within ± 0.5 C) and a circulating pump. Plastic pots measuring 21 cm in diameter and 16.5 cm in depth were filled with autoclaved sandy soil and placed in the controlled temperature tank. Each pot had a tube for drainage.

The tomato seeds were sowed in sterile Saftblast® and 12 days later the seedlings were transplanted to the plastic pots. Twelve days after transplanting, the soil was inoculated by pipetting an egg suspension into a hole 0.5 cm in diameter and 2.0 cm in depth near each plant. The egg extraction was made according to the method developed by Hussey and Barker (46). Infected roots of the Floradade tomato were shaken in a solution of 0.53% of sodium hypochlorite (NaOCl) for 4 minutes dissolving the egg-mass matrices. The freed eggs passed through a USA standard test sieve, number 200, with openings of 75

micrometers and were collected in another sieve, number 400, with openings of 38 micrometers.

The greenhouse experiments were performed in the same sequence in which they are described below.

Preliminary Screening Test at Two Temperatures

The main purpose of this experiment was to verify under controlled soil temperature the resistance to root-knot nematodes of lines 11-5 and 12-1, which were derived from L. peruvianum var. dentatum PI 129149. These lines had been previously screened at the AREC, Bradenton, Florida, in raised greenhouse beds. Additional PI accessions of L. peruvianum--126946, 251312, 365951, and 4 L. exculentum cultivars, Floradade, Nemared, Nematex, and Patriot were included in the experiment.

One to 8 pots with 4 seedlings of each entry were inoculated on November 2, 1978, with M. incognita-165 or M. javanica-181. One aliquot of 2,500 eggs was applied into holes around each plant. M. incognita-165 was originally collected in Gilchrist County, Florida. It has been studied morphometrically and by host differential tests by Lopez Chaves (52) and it has been shown to belong to race 1 of M. incognita. M. javanica-181 was originally collected in Columbia County, Florida.

The egg mass index (0--no egg mass, 1--1 to 2, 2--3 to 10, 3--11 to 30, 4--31 to 100, and 5--more than 100 egg masses per plant) (74, 76) and the gall diameter in millimeters were determined for each plant 28 days after inoculation. The controlled temperature tanks were maintained either at 25 or 32.5 C for 28 days. During the incubation period the greenhouse air temperature ranged from 19 to 28 C.

Effect of Inoculum Densities

The effect of 5 inoculum densities on 4 tomato accessions exposed to 2 temperatures and 2 species of Meloidogyne was studied in this experiment. Three L. esculentum cultivars, Floradade, Nematex, and Patriot, and one L. peruvianum PI 129149 (line 11-5) were used. Five inoculum densities were used: 20, 100, 200, 1,000, and 2,000 eggs per plant of M. incognita-165 or M. javanica-181. The 5 most uniform seedlings among the 10 seedlings transplanted per pot were selected at the time of inoculation. Twenty plants of each accession were inoculated with each inoculum level for each nematode species. Ten of them were grown at 25 C and 10 at 32.5 C in the controlled temperature tanks. After 28 days the plants were removed, and the roots were washed and examined for egg masses. During the experiment the greenhouse air temperature ranged from 18 to 25 C.

The data from the plants grown at 32.5 C were analyzed using the general linear models procedure in the Statistical Analyses System (SAS) at Northeast Regional Data Center (NERDC), Gainesville, Florida.

Preliminary Screening at Ambient Temperature

The objective of this test was to prescreen 91 tomato accessions at ambient temperature in order to eliminate the susceptible ones and to expose only the resistant ones in a subsequent test at a higher temperature. Twenty seedlings of the cultivar Floradade were used as a susceptible check while 4 seedlings of the following accessions were tested: the L. esculentum cultivars Anahu, Atkinson, Auburn 76FMN, Healani, Heinz 2990, Kewalo, Marsol, Monita, Piersol, Rossol, 74T2,

PI numbers 263710, 263711, 263721, 265955, 319695, 341128, 375937, and 376072; the L. peruvianum var. dentatum PI numbers 126435, 126439, 126441, 126928 to 126930, 126935, 126944 to 126946, 126946 (selfed), 127830 to 127832, 128643, 128645 to 128651, 128653 to 128657, 128659 to 128661, 128663, 129145, 129146, 129149, 129152, 143679, 212407, 246585, 246586, 251306 to 251311, 251314, 266375, 266376, 270435, 298933, 303814, 306811, 365968, 365969; the L. peruvianum var. humifusum PI accession 365951; L. cheesmanii var. minor PI accession 305896; and the L. esculentum x L. peruvianum PI accessions 298934 (lines 1 to 5), 306812, 306812 (lines 1 to 4), 306813 (lines 2 to 4), 306814 (lines 4 and 5).

Plastic flats were used with autoclaved soil, and the seedlings were inoculated with an egg suspension of M. incognita-165 averaging 750 eggs per plant. The flats were exposed at ambient temperature and the soil temperature in the flats ranged from 19 to 24 C during the experiment span. The greenhouse air temperature ranged, in this same period, from 18 to 25 C. The seedlings were evaluated for gall index (74, 76) 31 days after inoculation.

Screening at High Temperature

Fifty-four tomato accessions selected in the preliminary screening test at ambient temperature and 17 lines of the PI 129149 selected previously at the AREC, Bradenton, Florida, were included in the present screening experiment at high temperature. The following tomato accessions were used: the L. esculentum Anahu, Atkinson, Auburn 76FMN, Floradade, Healani, Kewalo, Marsol, Monita, Piersol, Rossol, 74T2, PI

265955, 298934-5, 319695, and 341128; the L. peruvianum var. dentatum PI numbers 126441, 126928 to 126930, 126935, 126944 to 126946, 127830, 128643, 128645 to 128651, 128654 to 128657, 128659, 128661, 129146, 129149, 129152, 143679, 212407, 246585, 246586, 251306, 251310, 251311, 266375, 266376, 303814, 303811; the lines derived from PI 129149 (from Bradenton), SN6-R3-5, SN9-R4-1, SN9-R4-2, SN9-R4-4, SN11-R2-1, SN11-R2-2, SN11-R2-5, SN12-R1-4 to -7, SN12-R2-1, SN12-R2-2, SN12-R2-5 to -7, and SN13-R2-9; and the L. esculentum x L. peruvianum crosses, PI 306812 (line 2), 306813 (line 4), and 306814 (line 5).

Ten plants of each line (grown in 2 pots) were inoculated on March 12, 1979, using 200 eggs per plant of M. incognita-165. After 28 days at 32.5 C in the controlled temperature tanks the plants were removed. The roots were washed and the number of egg masses per plant were counted. During the experiment the greenhouse air temperature ranged from 20 to 30 C.

Statistical analyses were computed using the Analyses of Variance (ANOVA) procedure (SAS) at NERDC, Gainesville, Florida.

Effect of Thermoperiodism

Two experiments were conducted in order to determine the effect of thermoperiodism on the resistance of tomatoes to root-knot nematode, M. incognita. In the first experiment only L. peruvianum lines were used. In the second a few L. esculentum cultivars were included.

In the first experiment PI 306811 and PI 129149 (lines SN9-R4-4, SN11-R2-2, and SN12-R2-1) were used. Ten seedlings of each accession were inoculated with 200 eggs of M. incognita-165 per plant on January 23, 1979.

The soil temperature was held continuously at 32.5 C in the controlled temperature tank. Ten seedlings of the same 4 lines were also inoculated with the same number of eggs of the same nematode population. These plants were exposed to diurnal temperature changes, 12 hours in the controlled temperature tank at 32.5 C (day) and 12 hours outside the tank at ambient temperature (19-25 C, night). After 28 days the number of egg masses per plant under both conditions was counted. During the experiment the greenhouse air temperature ranged from 19 to 27 C.

In the second experiment Floradade, Nematex-22, Patriot-17, Piersol, the F_1 of Floradade x Nematex-22, PI 266376, and PI 129149 (lines SN9-R4-4 and 11-5-16x43) were tested. Nematex-22 and PI 129149-11-5-16x43 are progenies from selections made in a preliminary screening in December, 1978. Ten seedlings of each accession were inoculated with 200 eggs of M. incognita-165 on May 8, 1979. This experiment was also exposed to the diurnal temperature changes described in the previous paragraph. In this test the air temperature in the greenhouse ranged from 19 to 24 C (night) and 24 to 30 C (day).

Statistical analyses of the second experiment were computed using the ANOVA procedure (SAS) at NERDC, Gainesville, Florida.

Effect of Length of Initial Exposure Time to Different Temperatures

Seedlings of Nematex-22, Floradade, and the line SN9-R4-4 (L. peruvianum PI 129149) were inoculated with 200 eggs of M. incognita-165 on May 8, 1979. The plants were grown in the controlled temperature tanks for various numbers of days at 32.5 C and then to 25 C for a

number of days (Table 9), or they were started at 25 C and transferred to the 32.5 C tank for the remaining days. Ten seedlings per treatment were used. During the incubation period the greenhouse air temperature ranged from 19 to 30 C.

The number of egg masses produced per plant were counted 30 days after inoculation, and the data obtained submitted to statistical analyses using the ANOVA procedure (SAS) at NERDC, Gainesville, Florida.

Effect of Races of M. incognita

In this experiment 9 tomato accessions were inoculated with 2 races of M. incognita to determine their effect on these tomato accessions at high temperature. The accessions were Floradade, Nematex-22, Patriot, Piersol, 74T2, the F_1 of Floradade x Nematex-22, PI 129149 lines 11-5-16x43 and SN9-R4-4, and PI 266376. Ten seedlings of each accession were inoculated with 200 eggs per plant of M. incognita-165 or M. incognita-Br. Meloidogyne incognita-165 belongs to race 1 and was originally collected at Gilchrist County, Florida. Meloidogyne incognita-Br was collected at AREC, Bradenton, Florida, and its identification was based on female perineal patterns, larval length (32), and host differential responses (65, 74, 76). It appears to belong to race 4 of M. incognita because it infected the cotton cultivar Deltapine 16 (egg mass index of 4) and the tobacco cultivar NC 95 (egg mass index of 5) (65, 66, 74, 76). However, "race 4" could be a mixture of race 2 and 3 since it has not been back tested to cotton and tobacco. During the experiment the greenhouse air temperature ranged from 19 to 30 C. The number of egg masses produced per plant was counted after 28 days of

incubation at 32.5 C in the controlled temperature tank. The data obtained were statistically analyzed using the ANOVA procedure (SAS) at NERDC, Gainesville, Florida.

Advanced Screening at High Temperature

Floradade, Chonto, and seeds from screened plants of several cultivars and PI accessions in the previous tests were put in this experiment. The selections used were the L. esculentum lines Anahu-1, Atkinson-1, Auburn 76FMN-1, Kewalo-1, Marsol-1, Monita-1, Nematex-22-1, Nematex-22-5, Piersol-1, Rossol-1, 74T2-1, 265955-1, 319695-1, 341128-1; the L. peruvianum var. dentatum lines 126441-1x2, 126928-1x2, 126935-1, 126941-1, 126945-1x2, 126946-1x2, 127830-1x2, 128645-1x2, 128646-1x2, 128649-1x2, 128651-1x2, 128656-1x2, 129146, 129149 (lines SN9-R4-2-1x2, SN9-R4-4-1x2, SN12-R1-7-1x2), 129149 (lines 11-5-16x43 and 11-5-16-selfed), and 246586-1, 251306-1x2, 266376-1x3, 306811-3x4; and the L. esculentum x L. peruvianum line 306814-1-1x2. Ten seedlings of each line were inoculated with an egg suspension of 200 eggs per plant of M. incognita-165 on August 19, 1979, and maintained in controlled temperature tanks at 32.5 C for 28 days. Then, the plants were removed, the roots were washed, and the number of egg masses per plant counted. During the experiment the greenhouse air temperature ranged from 19 to 32 C. The statistical analysis of the data used the ANOVA procedure (SAS) at NERDC, Gainesville, Florida.

Field Experiments

Field experiments were planted at the AREC, Bradenton, Florida, during March and September of 1978 and March of 1979. The following

cultivars and F_1 hybrids were planted in all 3 experiments: Floradade and Walter PF (*Mi*$^+$), Patriot and 74T2 (*Mi*), Walter PF x Patriot, and Walter PF x 74T2.

During March and September of 1978, seedlings were transplanted to field plots which were naturally infested with *M. incognita*, using a randomized block design with 4 replications. Ten seedlings of each cultivar were transplanted per plot on March 17, 1978, and on September 13, 1978. At the end of each crop season (June 12 and December 12, 1978) the plants were uprooted, the roots were washed, and a gall index from 0 (no galls) to 5 (more than 100 galls) was given to each plant. The soil temperature at 10 cm depth during the growing period (March/June) ranged from 14 to 32 C and during September/December ranged from 17 to 33 C.

In the March, 1979, experiment a full-bed mulch system was applied using polyethylene film. The field was prepared with 670 kg/ha of superphosphate containing 5.6 kg of fritted trace elements. After beds 0.25 m high by 0.70 m wide were formed, 1,680 kg/ha of 18-0-25 fertilizer was placed in bands on the shoulders (top edges) of each bed prior the mulching. Three weeks before transplanting, the field was fumigated by injecting 392 kg/ha of Dowfume MC-33® (methyl bromide 66% and chloropicrin 33%) into the beds. Irrigation was supplied by a seep ditch system in which seven 1.37 m beds were separated by a water ditch. The soil temperature at 10 cm depth at the AREC, Bradenton, during the crop growing period ranged from 20 to 37 C.

The experiment was arranged in a strip-plot design with 4 replications. Each subplot had the dimensions of 2.70 by 1.37 m. Six tomato seedlings were transplanted at 0.45 m spacing in each subplot on March 15. In each plot, one subplot was inoculated and another was

not. Each plant received an egg suspension containing 10,000 eggs which was applied with a pipette.

The fruits were harvested two times, on June 7 and 22. The number and weight of fruits were recorded. At the last harvest two plants in each subplot were pulled out and a root-knot gall index of 0 (no galls) to 5 (more than 100 galls) was given to each plant.

The reduction of yield due to nematode infestation, and the number, weight, and mean weight of fruits obtained in this experiment were submitted to statistical analyses using the ANOVA procedure (SAS) at NERDC, Gainesville, Florida.

RESULTS AND DISCUSSION

Greenhouse Experiments

Preliminary Screening Test at Two Temperatures

The number of egg masses observed on roots of 9 tomato accessions inoculated either with M. incognita-165 or M. javanica-181 incubated at 25 or 32.5 C are shown in Tables 1 and 2. There was a "breakdown" of resistance to M. incognita or M. javanica at 32.5 C in the cultivars Nematex and Patriot, both possessing Mi gene, and in PI 129149-11-5 and PI 129149-12-1. Floradade, Nemared, PI 126946, and PI 251312 had a high number of egg masses at both temperatures for both species. PI 365951 had a low egg mass index at 25 C when inoculated with M. javanica, but not with M. incognita. The low number of egg masses produced at 25 C in Nematex inoculated with M. incognita or M. javanica and in Patriot inoculated with M. incognita may be accounted for by small variations at the cellular level of some resistance mechanism, such as chlorogenic acid (45) or ascorbic acid concentration (2), which may have allowed the development of a small number of larvae after inoculation with 2,500 eggs per plant. These results agree with those of Zacheo et al. (92) who also found that cultivars possessing the Mi gene produced mature females after inoculation with 1,000 second stage larvae of M. incognita and incubation at 25-27 C. The higher number of egg masses of M. incognita or M. javanica

Table 1. Egg mass indices observed on 9 tomato accessions inoculated with 2,500 eggs of M. incognita-165 per plant and incubated at 25 or 32.5 C.

Cultivar or PI No.	Incubation temperature			
	25 C		32.5 C	
	Egg mass index[a]	Number of plants	Egg mass index[a]	Number of plants
Floradade	5.00	20	5.00	16
Nematex	0.15	20	4.44	16
Patriot	0.50	4	5.00	16
Nemared	4.05	20	5.00	12
126946	4.75	4	5.00	4
251312	5.00	4	4.50	4
365951	4.25	4	5.00	12
129149-11-5	0.00	20	3.71	28
129149-12-1	2.25	4	5.00	7

[a]Egg mass index: 0--0 egg mass per plant, 1--1 or 2, 2--3 to 10, 3--11 to 30, 4--31 to 100, and 5--more than 100 egg masses per plant.

Table 2. Egg mass indices observed on 9 tomato accessions inoculated with 2,500 eggs of M. javanica-181 per plant and incubated at 25 or 32.5 C.

Cultivar or PI No.	Incubation temperature			
	25 C		32.5 C	
	Egg mass index[a]	Number of plants	Egg mass index[a]	Number of plants
Floradade	5.00	20	5.00	20
Nematex	0.54	24	4.62	16
Patriot	0.00	4	5.00	16
Nemared	3.53	15	5.00	12
126946	5.00	12	5.00	5
251312	5.00	12	5.00	5
365951	1.12	16	4.50	4
129149-11-5	0.50	12	4.44	32
129149-12-1	4.00	4	4.75	16

[a]Egg mass index: 0--0 egg mass per plant, 1--1 or 2, 2--3 to 10, 3-11 to 30, 4--31 to 100, and 5--more than 100 egg masses per plant.

produced by Nemared at 25 C is difficult to explain. It may be that the Nemared source used in this experiment was not a pure line.

All cultivars and PI's had a high number of egg masses at 32.5 C, but the diameter of the galls was smaller in Nematex, PI 129149-11-5, PI 365951, PI 251312, PI 129946, and PI 129149-12-1 than in the susceptible cultivar Floradade (Table 3). According to Dropkin (24) there is a high positive correlation between gall size and the number of larvae in the gall. The results presented here suggest that the inoculum applied was excessive and that using an appropriate inoculum density is important when screening tomatoes at high temperature. Another experiment was necessary to investigate the effect of inoculum levels and to improve the technique of assessing the resistance to root-knot nematodes at 32.5 C.

One plant from Nematex and 10 plants from PI 129149-11-5 had the lowest ratings among all the plants in this test. Seeds were saved from these selections for future experiments. Of the 10 PI 129149-11-5 plants, one had an egg mass index of 2 (identified as 129149-11-5-16) and 9 had an egg mass index of 3. The Nematex plant (identified as Nematex-22) had an egg mass index of 3.

Effect of Inoculum Densities

The number of egg masses observed on 4 tomato accessions after inoculation with either *M. incognita*-165 or *M. javanica*-181 at 5 different inoculum levels and 2 different temperatures is presented in

Table 3. Gall diameter (mm) observed on roots of 9 tomato accessions inoculated with 2,500 eggs per plant of M. incognita-165 or M. javanica-181 and incubated at 32.5 C.

Cultivar or PI No.	Gall diameter (mm)	
	M. incognita-165	M. javanica-181
Floradade	2.2	1.9
Patriot	2.0	2.1
Nemared	1.7	1.3
129149-12-1	1.1	1.3
129946	1.0	0.7
251312	1.0	0.7
365951	0.8	0.8
129149-11-5	0.6	0.6
Nematex	0.5	1.4

Figures 1 and 2. The number of egg masses recorded for the susceptible cultivar, Floradade, was very similar to each inoculum density of both species of Meloidogyne at both temperatures. Compared to Floradade, a lower number of egg masses was found at 25 C on the 2 tomato lines carrying the Mi gene, Nematex, Patriot, and on PI 129149-11-5. At 25 C all 4 tomato accessions produced slightly more egg masses after inoculation with M. javanica than with M. incognita. There was also a "breakdown" at 32.5 C in the resistance of the 2 tomato accessions carrying the Mi gene and in PI 129149-11-5.

It can be observed that as the initial inoculum increased a higher number of egg masses was produced in all the cultivars and PI lines at 32.5 C for M. incognita and M. javanica (Figures 3 and 4). When the number of egg masses exceeded 100, it was recorded as > 100, following the commonly adopted procedure for rating galls and egg masses (65, 74, 76). There were no significant differences in the number of egg masses produced by Patriot, Nematex, and PI 129149-11-5 when inoculated either with 100 or 200 eggs per plant of M. incognita-165 at 32.5 C. However, these 3 tomato accessions inoculated with 200 eggs produced significantly fewer egg masses than were observed on the cultivar Floradade at half the initial inoculum concentration (Figure 3).

The number of egg masses of M. javanica produced by Floradade and Patriot with an inoculum level of 200 eggs per plant did not differ statistically at 32.5 C. Nematex and PI 129149-11-5 produced a similar

Figure 1. Response of 4 tomato accessions to 5 initial inoculum densities of M. incognita-165 at soil temperatures of 25 and 32.5 C determined by egg mass counts 30 days after inoculation.

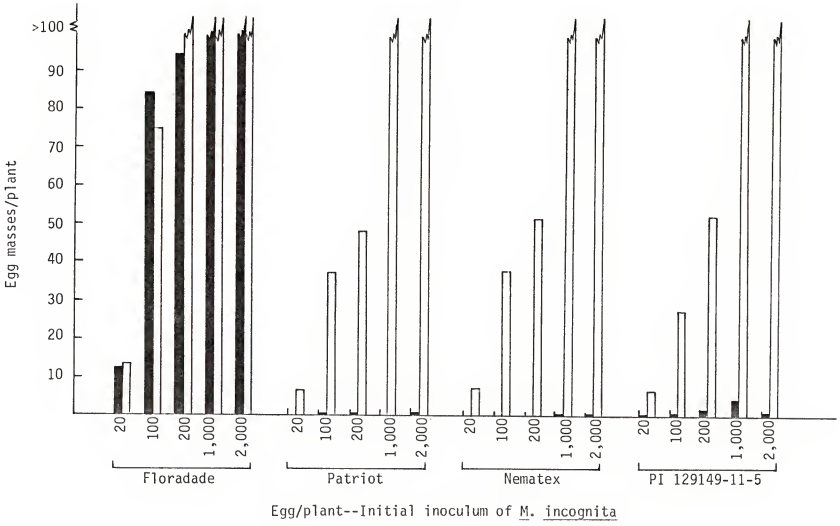

25 C
32.5 C
Egg masses/plant
>100
90
80
70
60
50
40
30
20
10
20
100
200
1,000
2,000
Floradade
Patriot
Nematex
PI 129149-11-5
Egg/plant--Initial inoculum of M. incognita

Figure 2. Response of 4 tomato accessions to 5 initial inoculum densities of M. javanica-181 at soil temperatures of 25 and 32.5 C determined by egg mass counts 30 days after inoculation.

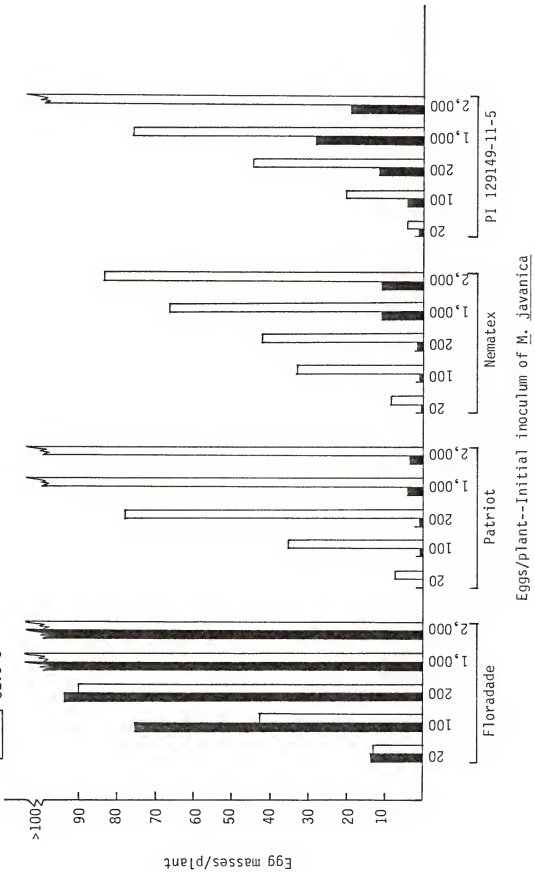

25 C
32.5 C
Egg masses/plant
>100
90
80
70
60
50
40
30
20
10
Floradade
Patriot
Nematex
PI 129149-11-5
20
100
200
1,000
2,000
Eggs/plant--Initial inoculum of M. javanica

Figure 3. Response of 4 tomato accessions to 5 initial inoculum densities of M. incognita-165 at a soil temperature of 32.5 C determined by egg mass counts 30 days after inoculation.

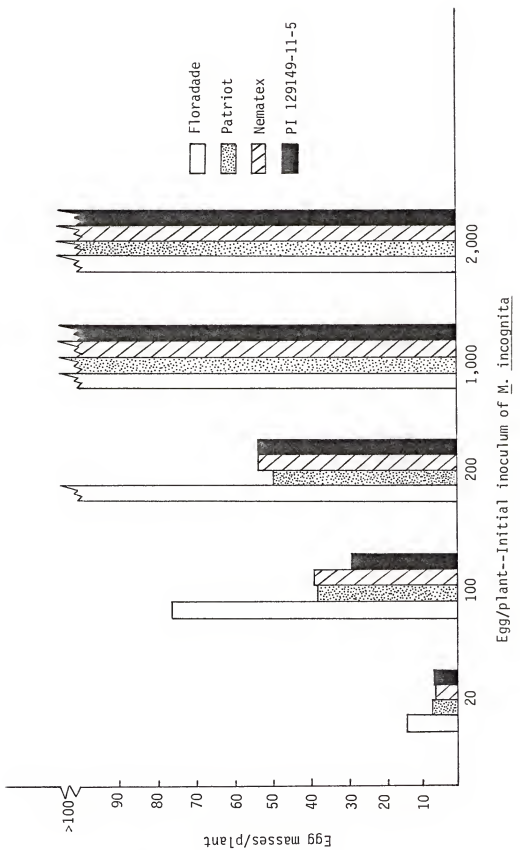
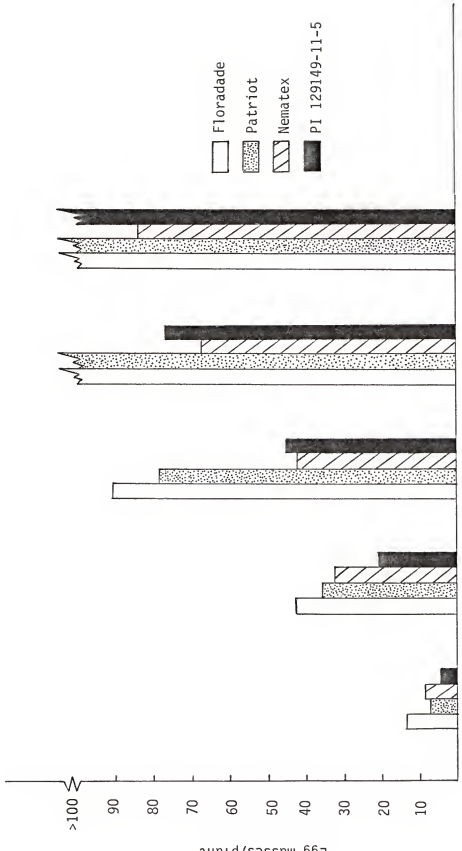

Floradade
Patriot
Nematex
PI 129149-11-5
Egg masses/plant
>100
90
80
70
60
50
40
30
20
10
20
100
200
1,000
2,000
Egg/plant--Initial inoculum of M. incognita

Figure 4. Response of 4 tomato accessions to 5 initial inoculum densities of M. javanica-181 at a soil temperature of 32.5 C determined by counts of egg masses 30 days after inoculation.


Floradade
Patriot
Nematex
PI 129149-11-5
Egg masses/plant
>100
90
80
70
60
50
40
30
20
10
20
100
200
1,000
2,000
Egg/plant--Initial inoculum of M. javanica

number of egg masses and differed significantly from Floradade and Patriot.

The concept of host efficiency (19) expresses the several plant-nematode relationships which comprise plant resistance. According to this concept, if the nematode reproduction is diminished, the plant is showing resistance. The degree to which the reproduction is affected determines the degree of resistance. Wallace (84) indicated that "in breeding plants for resistance, and in assessing the relative resistance of plant varieties, the rate of reproduction is the basic criterion. There are immune plants on which a particular nematode cannot reproduce at all, and there are susceptible ones on which it reproduces well. Between these extremes there is a spectrum of degree of resistance" (p. 75). This point of view has been adopted by other nematologists (76, 19). It appears that the immunity, resistance, or susceptibility of a host can be assessed by establishing the relationship between the initial inoculum density and the final number of egg masses produced after one life cycle has occurred under controlled temperature conditions. With this view in mind the data in Figures 3 and 4 can be interpreted to mean that an inoculum level of 200 eggs per plant is the best level for screening tomatoes at 32.5 C. At this level, individual egg masses can be observed and the degree of resistance assessed.

It is suggested that the counting of individual egg masses represents a major advance in the improvement of plant breeding technique for selection of resistance to root-knot nematodes. Counts of individual egg masses are similar in importance to counts of individual colonies in bacteriological studies. Quantitative nematode resistance can be based on this parameter.

In the present test Nematex, Patriot, and PI 129149-11-5 showed less than 50% of the reproduction observed in Floradade when inoculated with 200 eggs of M. incognita per plant. Similarly, Nematex-22 and PI 129149-11-5 showed less than 50% of the reproduction observed in Floradade when inoculated with 200 eggs of M. javanica per plant. According to Taylor and Sasser's classification of resistance (76), these lines could be considered as slightly resistant at high temperatures.

Preliminary Screening at Ambient Temperature

The results obtained from tomatoes grown in inoculated soil flats and exposed to ambient temperature are presented in Table 4. Among 91 Lycopersicon accessions 15 showed more than 100 galls per plant and 55 had no galls. Floradade and Heinz 2990 do not carry the Mi gene. Atkinson, a cultivar possessing the Mi gene, had 3 plants without galls and 1 with a gall index of 5, indicating genetic or mechanical impurity. In the PI 263711, however, 4 different gall indices were observed. In the L. esculentum x L. peruvianum group, the presence of gall indices of 0 and 5 among selections of PI 298934 indicated great genetic variability for resistance in the L. peruvianum ancestor. This is not an unexpected result in light of the fact that most L. peruvianum are cross-pollinated.

Fifty-four tomato accessions were selected for the following screening at high temperature.

Table 4. Evaluation of the resistance to M. incognita-165 of 91 tomato accessions grown in flats which were inoculated with 750 eggs per plant at 18-25 C.

Cultivar or PI No.	Gall index[a]	Cultivar or PI No.	Gall index[a]
L. esculentum		126439	2.50
Floradade	5.00	126441	0.00
Anahu	0.00	126928	0.00
Atkinson	1.25	126929	0.00
Auburn 76FMN	0.00	126930	0.00
Healani	0.00	126935	0.00
Heinz 2990	5.00	126944	0.00
Kewalo	0.00	126945	0.00
Marsol	0.00	126946	0.00
Monita	0.00	126946-selfed	0.00
Piersol	0.00	127830	0.00
Rossol	0.00	127831	2.00
74T2	0.00	127832	3.50
263710	5.00	128643	0.00
263711	2.75	128645	0.00
263721	5.00	128646	0.00
265955	0.00	128647	0.00
319695	0.00	128648	0.00
341128	0.00	128649	0.00
375937	5.00	128650	0.00
376072	5.00	128651	0.00
L. peruvianum var. dentatum		128653	1.25
126435	3.25	128654	0.00

Table 4. continued

Cultivar or PI No.	Gall index[a]	Cultivar or PI No.	Gall index[a]
128655	0.00	270435	5.00
128656	0.00	298933	5.00
128657	0.00	303814	0.00
128659	0.00	306811	0.00
128660	1.50	365968	4.50
128661	0.00	365969	5.00
128663	0.75	*L. peruvianum* var. *humifusum*	
129145	1.50	365951	1.75
129146	0.00	*L. cheesmanii* var. *minor*	
129149	0.00	305896	4.50
129152	0.00	*L. esculentum* x *L. peruvianum*	
143679	0.00	298934-1	5.00
212407	0.00	298934-2	5.00
246585	3.25	298934-3	5.00
246586	0.00	298934-4	5.00
251306	0.00	298934-5	0.00
251307	5.00	306812	3.75
251308	5.00	306812-1	2.00
251309	3.50	306812-2	0.00
251310	0.00	306812-3	2.50
251311	0.00	306812-4	2.75
251314	3.50	306813-2	0.00
266375	0.00		
266376	0.00		

Table 4. continued

Cultivar or PI No.	Gall index[a]	Cultivar or PI No.	Gall index[a]
306813-3	0.00	306814-4	0.25
306813-4	0.00	306814-5	0.00

[a]Gall index scale: 0 (no gall) and 5 (more than 100 galls/plant). Four plants of each tomato line were inoculated, except for Floradade, in which case 20 plants were inoculated.

Screening at High Temperature

The number of egg masses observed on 72 tomato accessions held at 32.5 C for 28 days after inoculation with 200 eggs of M. incognita-165 per plant is shown in Table 5. Floradade had an average of 80.9 egg masses per plant, which was the highest number observed in this test. The resistance observed among the different tomato accessions ranged from 55.6 to 8 egg masses per plant. According to Taylor and Sasser's proposed classification (76), 4 groups of plants could be distinguished among the 72 cultivars and PI accessions in Table 5:

1. susceptible, represented by Floradade;
2. moderately susceptible, including the PI's whose means are followed by letter b;
3. slightly resistant, including cultivars and PI accessions followed by the letters p and l;
4. moderately resistant, including cultivars and PI accessions followed by the letter y.

It has been demonstrated that at 25-27 C different concentrations of ascorbic acid (2) are produced by different tomato cultivars having the Mi gene after inoculation with M. incognita. The higher the induced concentration of this compound, the higher the resistance of the cultivar. One possible explanation for the variation in resistance presented by tomato accessions of L. esculentum carrying the Mi gene (Table 5), such as Healani with 39.0 egg masses per plant, Rossol with 17.7, and 74T2 with 8.0, may be that under high temperature those cultivars produce different concentrations of one or more chemical inhibitors to larval development in root-knot nematode. Such antibiosis could be under genetical control.

Table 5. Number of egg masses observed on 72 tomato accessions inoculated with 200 eggs per plant of *M. incognita*-165 and maintained at 32.5 C for 28 days.

Cultivar or PI No.	Mean number of egg masses/plant[a]
Floradade	80.9 a
246585	55.6 b
298934-5	53.5 bc
303814	53.0 bcd
306813-4	51.7 bcde
143679	49.5 bcdef
246586	49.1 bcdefg
128657	48.2 bcdefg
212407	47.9 bcdefg
251310	47.7 bcdefgh
128654	47.0 bcdefghi
306811	44.5 bcdefghij
129149-SN11-R2-5	44.3 bcdefghij
129149	43.6 bcdefghijk
128655	40.6 cdefghijkl
251311	40.4 cdefghijkl
129152	40.3 cdefghijkl
129149-SN12-R2-5	40.1 cdefghijkl
128650	39.9 cdefghijkl
126930	39.8 cdefghijklm
Healani	39.0 cdefghijklmn
126929	38.6 cdefghijklmno
126935	38.3 defghijklmno
266375	38.3 defghijklmno

Table 5. continued

Cultivar or PI No.	Mean number of egg masses/plant[a]
128643	38.2 defghijklmno
129149-SN12-R2-2	37.7 efghijklmnop
128647	37.4 efghijklmnop
129149-SN11-R2-1	37.0 efghijklmnop
129149-SN9-R4-1	36.9 efghijklmnop
128649	36.5 fghijklmnopq
128659	36.0 fghijklmnopq
128661	35.7 fghijklmnopq
265955	34.6 r fghijklmnopq
129149-SN12-R2-7	34.0 r ghijklmnopq
129149-SN12-R1-4	33.7 rs ghijklmnopq
129146	33.5 rs ghijklmnopq
129149-SN6-R3-5	33.3 rs ghijklmnopq
128656	32.7 rst hijklmnopq
129149-SN12-R2-6	32.6 rstu ijklmnopq
128648	32.1 rstu jklmnopq
129149-SN13-R2-9	31.5 rstuv jklmnopq
128651	31.2 rstuv jklmnopq
126944	31.0 rstuv jklmnopq
129149-SN11-R2-2	30.9 rstuv jklmnopq
129149-SN9-R4-2	30.7 rstuv jklmnopq
129149-SN12-R1-5	30.4 rstuv jklmnopq
251306	29.9 rstuv jklmnopq
128645	28.6 rstuvw klmnopq
129149-SN12-R2-1	28.3 rstuvw lmnopq

Table 5. continued

Cultivar or PI No.	Mean number of egg masses/plant[a]	
Kewalo	28.2 rstuvw	lmnopq
126928	28.0 rstuvw	lmnopq
Auburn 76FMN	27.9 rstuvwx	lmnopq
127830	27.6 rstuvwx	lmnopq
Anahu	26.7 rstuvwx	lmnopq
341128	25.9 rstuvwx	lmnopq
129149-SN12-R1-6	25.5 rstuvwx	lmnopq
126946	24.6 rstuvwx	mnopq
319695	24.5 rstuvwx	nopq
306812-2	23.9 rstuvwx	nopq
128646	23.7 rstuvwx	opq
129149-SN12-R1-7	22.8 rstuvwx	pq
129149-SN9-R4-4	21.7 rstuvwxy	q
126441	21.4 rstuvwxy	q
126945	20.4 rstuvwxy	
266376	18.8 stuvwxy	
Rossol	17.7 tuvwxy	
306814-5	17.4 uvwxy	
Monita	16.6 vwxy	
Piersol	13.8 wxy	
Atkinson	13.7 wxy	
Marsol	13.1 xy	
74T2	8.0 y	

[a]Means followed by the same letter are not significantly different by Duncan's multiple range test (alpha = 0.05).

In this screening test, individual plants having a low number of egg masses were selected. The number of plants saved per cultivar or PI accession and the number of egg masses observed on each plant are shown in Table 6. These plants were sib-mated or self-pollinated in order to obtain seeds to be tested in the advanced screening test under controlled high temperature.

Effect of Thermoperiodism

The effect of high day (32.5 C) and lower night (20-25 C) soil temperatures on the reproduction of M. incognita-165 in the exploratory experiment is shown in Table 7. This experiment indicated that resistance to root-knot nematode of L. peruvianum PI's 306811, 129149-SN9-R4-4, 129149-SN11-R2-2, and 129149-SN12-R2-1 was greater under differential temperature conditions than when maintained at constant high temperature. In addition to the generally lower reproduction rating, 6 plants without egg masses were found in 3 of the lines subjected to diurnal changes in soil temperatures. This indicates that the second stage larvae were unable to develop and reach maturity under such thermoperiodic condition.

Results of the second thermoperiod test (Table 8) were similar to those observed in the exploratory test. All tomato accessions which were maintained continuously at a 32.5 C soil temperature exhibited a significantly higher number of egg masses than when exposed to diurnal soil temperature changes (32.5 day/22-25 C night). These results may explain why in many places the Mi gene for resistance to root-knot nematodes in tomato cultivars is operative even with high day temperatures. Thermoperiodism was described by Went (90) and is known to

Table 6. Number of plants saved and respective number of egg masses counted in the screening at high temperature.

Cultivar or PI No.	Number of plants saved	Number of egg masses/plant
Anahu	1	17
Atkinson	2	5-8
Auburn 76FMN	2	15-16
Kewalo	2	12-14
Marsol	2	7-8
Monita	1	11
Piersol	2	5-6
Rossol	2	11-12
74T2	4	3-3-4-4
126928	2	9-15
126935	1	4
1266441	2	5-7
126944	2	10-11
126945	2	10-13
126946	2	6-6
127830	2	15-18
128645	2	6-14
128646	2	9-14
128649	2	13-18
128651	2	2-16
128656	2	8-11
129146	2	9-16
129149-SN9-R4-2	2	12-13
129149-SN9-R4-4	2	12-16

Table 6. continued

Cultivar or PI No.	Number of plants saved	Number of egg masses/plant
129149-SN12-R1-7	2	12-13
246586	1	15
251306	2	12-13
265955	1	16
266376	2	3-3
306814-5	2	8-9
319695	2	5-10
341128	1	10

Table 7. Effect of thermoperiodism on resistance of lines of L. peruvianum var. dentatum inoculated with 200 eggs per plant of M. incognita-165.

Line or PI No.	Diurnal temperature C	Number of egg masses/plant	Number of plants with 0 egg mass[a]
Floradade	32.5	80.9	0
L. peruvianum var. dentatum:			
306811	32.5[b]	44.5	0
	32.5/20-25[c]	4.7	0
129149-SN9-R4-4	32.5	21.7	0
	32.5/20-25	1.7	3
129149-SN9-R4-4	32.5	30.9	0
	32.5/20-25	5.1	2
129149-SN12-R2-1	32.5	28.3	0
	32.5/20-25	3.3	1

[a]Among 10 plants.

[b]Constant temperatures of 32.5 C.

[c]Diurnal T--12 daytime hours at 32.5 C/12 night hours at 20-25 C.

Table 8. Effect of thermoperiodism on resistance to M. incognita-165 of 9 tomato accessions inoculated with 200 eggs per plant.

Cultivar or PI No.	Diurnal temperature C	Egg masses per plant[a]	Plants without egg masses[b]
Floradade	32.5	108.2 a	0
	32.5/22-25[c]	88.5 b	0
Nematex-22	32.5	16.0 a	0
	32.5/22-25	0.9 b	5
Patriot-17	32.5	19.8 a	0
	32.5/22-25	0.4 b	7
Floradade x Nematex (F_1)	32.5	41.0 a	0
	32.5/22-25	4.0 b	0
Piersol	32.5	32.9 a	0
	32.5/22-25	1.1 b	2
74T2	32.5	27.9 a	0
	32.5/22-25	3.2 b	0
129149-11-5-16x43	32.5	6.9 a	1
	32.5/22-25	0.3 b	8
129149-SN9-R4-4	32.5	24.9 a	0
	32.5/22-25	1.0 b	7
266376	32.5	23.7 a	0
	32.5/22-25	3.6 b	2

[a]Means followed by the same letter within each cultivar or PI are not significantly different by Duncan's multiple range test (alpha = 0.05).

[b]Among 10 plants.

[c]Diurnal T--12 daytime hours at 32.5 C/12 night hours at 22-25 C.

influence the growth of the tomato plant. It was not surprising to find that thermoperiodism strongly affects the reproduction of root-knot nematode in tomato roots. Night soil temperature seems to be more important than day temperature as a factor controlling tomato resistance to M. incognita-165. Although night soil temperatures under field conditions are normally lower than daytime soil temperatures, recent innovations in cultural practices such as the use of full-bed plastic mulch keep the soil temperature high for a longer time after dark than would be the case with uncovered soil. Plastic mulch may maintain soil temperature above the limit for normal function of the Mi gene.

When nine cultivars and PI assessions were screened at a continuous temperature of 32.5 C, Floradade had the highest nematode reproduction (Table 9). Different levels of resistance to M. incognita-165 were observed among the cultivars carrying the Mi gene. In order of decreasing numbers of egg masses per plant, these were Floradade x Nematex-22 (F_1), Piersol, 74T2, Patriot-17, and Nematex. L. peruvianum 129149-11-5-16x43 (selected sib) had the lowest nematode reproduction in the test, and it was the first line grown continuously at 32.5 C to have a plant without any egg mass production (Table 8).

The same 9 cultivars and PI accessions were also screened under diurnal changes in temperature (Table 9). Three different groups of tomato accessions were found. One included only the susceptible cultivar Floradade, which had 88.2 egg masses per plant. Another group included Floradade x Nematex-22 (F_1), 74T2, and PI 266376, which had mean egg masses ranging from 4 to 3.2. A third group included Piersol, PI 129149-SN9-R4-4, Nematex-22, Patriot-17, and PI 129149-11-5-16x43, which all had less than 1.2 egg masses per plant.

Table 9. Effect of continuous high soil temperature and alternated high/low soil temperatures on the resistance of 9 tomato accessions inoculated with 200 eggs per plant of M. incognita-165.

Cultivar or PI No.	Number of egg masses/plant[a]
A. Maintained continuously at 32.5 C	
Floradade	108.2 a
Floradade x Nematex-22 (F_1)	41.0 b
Piersol	32.9 bc
74T2	27.9 cd
129149-SN9-R4-4	24.9 cd
266376	23.7 cde
Patriot-17	19.8 de
Nematex-22	16.0 e
129149-11-5-16x43	6.9 f
B. Maintained for 12 hours at 32.5 C (day) and 12 hours at 22-25 C (night)	
Floradade	88.5 a
Floradade x Nematex-22 (F_1)	4.0 b
266376	3.6 b
74T2	3.2 b
Piersol	1.1 c
129149-SN9-R4-4	1.0 c
Nematex-22	0.9 c
Patriot-17	0.4 c
129149-11-5-16x43	0.3 c

[a]Means followed by the same letter in each group, A or B, are not significantly different by Duncan's multiple range test (alpha = 0.05).

Effect of the Length of Initial Exposure Time to Different Temperatures

The effects of various lengths of initial exposure time at 32.5 C on 3 tomato accessions inoculated with 200 eggs per plant of M. incognita-165 are shown in Table 10. Floradade had higher nematode reproduction than Nematex-22 and PI 129149-SN9-R4-4 in all temperature treatments. Nematex-22 and PI 129149-SN9-R4-4 maintained complete resistance with 0 days of 32.5 C treatment, receiving all 30 days at 25 C. In all treatments Nematex-22 exhibited a lower reproduction rating than the L. peruvianum selection of PI 129149.

The effect of various lengths of initial exposure time at 25 C on the same 3 tomato accessions mentioned above and inoculated with 200 eggs of M. incognita-165 are shown in Table 11. Floradade again had the highest nematode reproduction in all treatments. In all treatments except G, the number of egg masses produced on Nematex was higher than the number on PI 129149-SN9-R4-4. This was the reverse of the relationship observed in the treatments in Table 10.

Considering the 6 treatments under each initial temperature as 2 separate systems, system 1 exposed initially at 32.5 C and system 2 exposed initially at 25 C, it was observed that they were different with respect to nematode reproduction. Treatments G through L in system 2 had an average of 46.22 egg masses per plant versus 42.01 in treatments A through F in system 1.

The effects of the 6 lengths of initial exposure time at 32.5 C on the 3 tomato accessions are grouped by cultivar in Table 12. Floradade showed no differences among 4 of the treatments, but exhibited a lower number of egg masses in treatments D and E. Nematex-22 after

Table 10. Effect of various lengths of initial exposure time at 32.5 C on 3 tomato accessions inoculated with 200 eggs per plant of M. incognita-165.

Treatment	Prior days at 32.5 C	Subsequent days at 25 C	Cultivar of PI No.	Egg masses per plant[a]
A	0	30	Floradade	103.7 a
			129149-SN9-R4-4	0.0 b
			Nematex-22	0.0 b
B	3	27	Floradade	99.3 a
			129149-SN9-R4-4	4.9 b
			Nematex-22	1.3 c
C	6	24	Floradade	100.1 a
			129149-SN9-R4-4	29.6 b
			Nematex-22	6.7 c
D	9	21	Floradade	92.0 a
			129149-SN9-R4-4	21.1 b
			Nematex-22	6.9 c
E	12	18	Floradade	90.9 a
			129149-SN9-R4-4	38.1 b
			Nematex-22	12.5 c
F	30	0	Floradade	108.2 a
			129149-SN9-R4-4	24.9 b
			Nematex-22	16.0 c

[a]Means followed by the same letter within each treatment are not significantly different by Duncan's multiple range test (alpha = 0.05).

Table 11. Effect of various lengths of initial exposure time at 25 C on 3 tomato accessions inoculated with 200 eggs per plant of M. incognita-165.

Treatment	Prior days at 25 C	Subsequent days at 32.5 C	Cultivar or PI No.	Egg masses per plant[a]
G	0	30	Floradade	108.2 a
			129149-SN9-R9-4	24.9 b
			Nematex-22	16.0 c
H	3	27	Floradade	101.9 a
			Nematex-22	36.8 b
			129149-SN9-R4-4	19.0 c
I	6	24	Floradade	108.4 a
			Nematex-22	29.9 b
			129149-SN9-R4-4	26.0 c
J	9	21	Floradade	104.9 a
			Nematex-22	24.1 b
			129149-SN9-R9-4	6.8 c
K	12	18	Floradade	102.1 a
			Nematex-22	12.9 b
			129149-SN9-R4-4	6.3 c
L	30	0	Floradade	103.7 a
			Nematex-22	0.0 b
			129149-SN9-R4-4	0.0 b

[a]Means followed by the same letter within each treatment are not significantly different by Duncan's multiple range test (alpha = 0.05).

Table 12. Effect of various lengths of initial exposure time at 32.5 C on Floradade, Nematex-22, and PI 129149-SN9-R4-4, inoculated with 200 eggs per plant of M. incognita-165.

Cultivar or PI No.	Treatment	Prior days at 32.5 C	Subsequent at 25 C	Egg masses per plant[a]
Floradade	F	30	0	108.2 a
	A	0	30	103.7 a
	C	6	24	100.1 a
	B	3	27	99.3 a
	D	9	21	92.0 b
	E	12	18	90.9 b
Nematex-22	F	30	0	16.0 a
	E	12	18	12.5 a
	D	9	21	6.9 b
	C	6	24	6.7 b
	B	3	27	1.3 c
	A	0	30	0.0 d
129149-SN9-R4-4	E	12	18	38.1 a
	C	6	24	29.6 ab
	F	30	0	24.9 b
	D	9	21	21.1 b
	B	3	27	4.9 c
	A	0	30	0.0 d

[a]Means followed by the same letter within each cultivar or PI are not significantly different by Duncan's multiple range test (alpha = 0.05).

exposure to either 30 prior days or 12 prior days at 32.5 C did not show significant differences between these treatments. This suggests that only 12 days of exposure to soil temperature of 32.5 C is needed to determine the behavior of tomato accessions to root-knot nematode. By using only 12 days for the maximum initial exposure, one could increase the number of plants screened per tank.

PI 129149-SN9-R4-4 showed no differences when grown at 6, 9, or 30 prior days at 32.5 C. Plants of this PI grown for 12 prior days at 32.5 C produced a higher number of egg masses than the treatment at 30 prior days, but the 12 prior day treatment was not significantly different from the 6 prior day treatment at this soil temperature. This variation may be explained by the absence of prior selection for resistance to M. incognita-165 in this L. peruvianum PI and by the higher genetical variation resulting from the cross-pollination habit of this species. Plants of Nematex-22 and PI 129149-SN9-R4-4 exposed to 3 prior days at 32.5 C and held subsequently for 27 days at 25 C had significantly fewer egg masses than plants exposed to 6 or more days at 32.5 C.

The general tendency of the 3 tomato accessions for treatments A through F can be seen in Table 12. Floradade had high reproduction at all levels. Reproduction of the root-knot nematode in Nematex-22 and PI 129149-SN9-R4-4 was strongly influenced by initial exposure time to 32.5 C in the range of 0 to 12 days.

The effect of 6 lengths of initial exposure time at 25 C on 3 tomato accessions are shown in Table 13. Floradade in all treatments had a similar number of egg masses per plant. Nematex-22 had a higher number of egg masses when exposed to only 3 and 6 days at 25 C. PI

Table 13. Effect of various lengths of initial exposure time at 25 C on Floradade, Nematex-22, and PI 129149-SN9-R4-4, inoculated with 200 eggs per plant of M. incognita-165.

Cultivar or PI No.	Treatment	Prior days at 25 C	Subsequent at 32.5 C	Egg masses per plant[a]
Floradade	I	6	24	108.4 a
	G	0	30	108.2 a
	J	9	21	104.9 a
	L	30	0	103.7 a
	K	12	18	102.1 a
	H	3	27	101.9 a
Nematex-22	H	3	27	36.8 a
	I	6	24	29.9 ab
	J	9	21	24.1 b
	G	0	30	16.0 c
	K	12	18	12.9 c
	L	30	0	0.0 d
129149-SN9-R4-4	I	6	24	26.0 a
	G	0	30	24.9 ab
	H	3	27	19.0 b
	J	9	21	6.8 c
	K	12	18	6.3 c
	L	30	0	0.0 d

[a]Means followed by the same letter within each cultivar or PI are not significantly different by Duncan's multiple range test (alpha = 0.05).

129149-SN9-R4-4 had a high number of egg masses when exposed to only 0, 3, and 6 days at the low initial temperature of 25 C. The general tendency of the 3 tomato accessions for treatments G through L is shown in Table 13. Floradade presented little variation in nematode reproduction, but Nematex-22 and PI 129149-SN9-R4-4 tended to produce a lower number of egg masses as the initial exposure time at 25 C increased.

The results of the present study differ from those obtained by Dropkin (27). He worked with a different species of Meloidogyne and used the second stage larvae as his source of inoculum. In his work only the larvae that penetrated the roots during the first 2 or 3 days were permitted to develop because he washed the roots after this period and replanted the seedlings in fresh soil free of larvae. The present work used the egg suspension method described by Hussey and Barker (46) and avoided disturbing the seedlings after they had been transplanted into pots twelve days before inoculation. Also more treatments were included here. Dropkin (27) concluded that the 3 days after inoculation determine the course of the plant-nematode relationship. This period was extended to a period of 12 days in the present work in order to permit a quantitative evaluation of resistance in tomatoes when using inoculum densities of 200 eggs per plant of M. incognita.

It was surprising that plants of Nematex-22 exposed for 3, 6, or 9 days to 25 C and subsequently at 32.5 C had greater nematode reproduction than those exposed initially or continuously at 32.5 C. In the development of a host-parasite relationship "each action and reaction is a potential source of resistance" (62, p. 245). Similarly, each action and reaction in a resistant cultivar may be a potential

source of susceptibility. The nematode-plant relationship is a constant interaction in which temperature can influence both the plant and the nematode, but each has a different optimum (84). The combinations of temperature presented above were the ones that most favored the nematode, but not the plant, contributing to an increased reproduction rate in Nematex-22. The results can not be explained in terms of hatching and motility (14), considering that all treatments involving the susceptible cultivar produced consistently similar results.

Effect of Races of M. incognita

The effects of 2 races of M. incognita on each tomato cultivar or PI accessions studied are shown in Table 14. The number of egg masses produced on the susceptible cultivar Floradade, PI 266376, and PI 129149-SN9-R4-4, by race 1 or race 4 were not significantly different. The number of egg masses produced on all others--Nematex-22, Floradade x Nematex-22 (F_1), Patriot, Piersol, 74T2, and PI 129149-11-5-16x43--by race 4 was significantly higher than that produced by race 1. This indicated that race 4 is better adapted to these tomato hosts than is race 1. The effects of race 1 and 4 on 9 tomato genotypes are shown in Table 15. The L. peruvianum accessions had higher levels of resistance to race 4 than did the L. esculentum cultivars. PI 129149-11-5-16x43 had the lowest number of egg masses among the 9 tomato accessions when inoculated with race 1 or race 4 of M. incognita, and one plant of this line showed no galls when inoculated with race 1. These results may be explained in part by the fact that this L. peruvianum line had been selected previously for resistance to race 4 at

Table 14. Effect of 2 races of M. incognita on the number of egg masses produced on each of the 9 tomato accessions inoculated with 200 eggs per plant at 32.5 C.

M. incognita race number[a]	Cultivar or PI No.	Egg masses/plant[b]
	L. esculentum	
4	Floradade	102.5 a
1	Floradade	108.2 a
4	Nematex-22	54.2 a
1	Nematex-22	16.0 b
4	Floradade x Nematex-22 (F_1)	53.7 a
1	Floradade x Nematex-22 (F_1)	41.0 b
4	Patriot	46.2 a
1	Patriot	19.8 b
4	Piersol	60.5 a
1	Piersol	32.9 b
4	74T2	47.0 a
1	74T2	27.9 b
	L. peruvianum var. dentatum	
4	129149-11-5-16x43	14.9 a
1	129149-11-5-16x43	6.9 b
4	129149-SN9-R4-4	25.0 a
1	129149-SN9-R4-4	24.9 a
4	266376	29.7 a
1	266376	23.7 a

[a]M. incognita-165 (race 1) was collected originally at Gilchrist Co., Florida, and M. incognita-Br (race 4) at Bradenton, Florida.

[b]Means followed by the same letter within each cultivar or PI are not significantly different by Duncan's multiple range test (alpha = 0.05).

Table 15. Effect of 2 races of M. incognita on the number of egg masses produced on 9 tomato accessions inoculated with 200 eggs per plant and incubated at 32.5 C.

M. incognita	Cultivar or PI No.	Egg masses/plant[a]
Race 4	Floradade	102.5 a
	Piersol	60.5 b
	Nematex-22	54.2 b
	Floradade x Nematex-22 (F_1)	53.7 b
	74T2	47.0 b
	Patriot	46.2 b
	266376	29.7 c
	129149-SN9-R4-4	25.0 cd
	129149-11-5-16x43	14.9 d
Race 1	Floradade	108.2 a
	Floradade x Nematex-22 (F_1)	41.0 b
	Piersol	32.9 bc
	74T2	27.9 cd
	129149-SN9-R4-4	24.9 cd
	266376	23.7 cde
	Patriot	19.8 de
	Nematex-22	16.0 e
	129149-11-5-16x43	6.9 f

[a]Means followed by the same letter within each race of M. incognita are not significantly different by Duncan's multiple range test (alpha = 0.05).

the AREC at Bradenton and for race 1 at Gainesville. The phenotypic variation exhibited between and within these accessions may indicate that residual genetic variation is still available for selection of lines with higher resistance against both races.

These results suggest that a survey of races within the Meloidogyne species found in the Florida tomato growing areas would be helpful in breeding for resistance.

Advanced Screening at High Temperature

The responses of 40 tomato accessions held at 32.5 C for 28 days to inoculations with 200 eggs per plant of M. incognita-165 are presented in Table 16. The results indicated that selection for plants with a low number of egg masses made in the previous screening tests was effective. The progenies of these selections had mean values of less than 20 egg masses per plant. Five types of plant response to root-knot nematode were assessed: susceptible (Floradade), slightly resistant (Chonto, a cultivar from Colombia), moderately resistant (tomato accessions with means between 18.1 and 10.4 egg masses per plant), resistant (means between 9.8 and 2.0 egg masses per plant), and highly resistant (PI 129149-11-5-15 with a mean of 0.4 egg masses per plant). Five tomato selections produced plants with no egg masses (Table 16). Among these was PI 129149-11-5-16, which produced 7 plants without egg masses out of the 10 plants screened. Repeated attempts (from January to July, 1979) to self-pollinate PI 129149-11-5-16 resulted in 837 fruits. The vast majority was parthenocarpic, but 15 normal seeds were obtained from these fruits. These seeds were included in the test (Table 16), producing plants which may be

Table 16. Response of 40 tomato accessions held at 32.5 C for 28 days to inoculation with 200 eggs per plant of M. incognita-165.

Cultivar or Line	Egg masses/plant[a]	Plants without egg masses
Floradade	110.5 a	0
Chonto	49.6 b	0
129146-1	18.1 c	0
128651-1x2	17.5 c	0
246586-1	16.8 cd	0
Hot Set x Nematex-22 (F_1)	16.7 cd	0
Anahu-1	15.8 cde	0
266376-1x3	14.5 cdef	0
306811-3x4	13.8 cdefg	0
126945-1x2	13.6 cdefg	0
Rossol-1	13.1 cdefg	0
Marsol-1	13.1 cdefg	0
126944-1	12.9 cdefg	0
Auburn 76FMN-1	12.6 cdefgh	0
74T2-1	12.4 cdefgh	0
341128-1	12.0 cdefgh	0
Kewalo-1	11.5 defghi	0
127830-1x2	11.4 efghi	0
128649-1x2	10.9 efghi	0
265955-1x2	10.6 efghij	0
126946-1x2	10.4 efghij	0
128656-1x2	9.8 fghijk	0
251306-1x2	9.3 fghijk	0
319695-1	8.6 ghijk	0

Table 16. continued

Cultivar or Line	Egg masses/plant[a]	Plants without egg masses
Atkinson-1	8.2 l hijk	0
128645-1x2	7.4 l ijk	0
306814-1x2	7.3 lm ijk	0
129149-SN9-R4-4-1x2	6.4 lm jk	0
128646-1-1x2	6.0 lmn k	0
Nematex-22-1	5.9 lmn k	0
Piersol-1	5.6 lmn k	0
Monita-1	4.6 lmn	0
129149-SN12-R1-7-1x2	4.0 mno	0
Nematex-22-5	3.1 mno	0
126928-1x2	3.1 no	1
129149-11-5-16x43	3.0 no	0
126935-1	3.0 no	1
126441-1x2	2.9 no	1
129149-SN9-R4-2-1x2	2.0 o	2
129149-11-5-16 (selfed)	0.4 p	7

[a]Means followed by the same letter are not significantly different by Duncan's multiple range test (alpha = 0.05).

classified as immune to an inoculum concentration of 200 eggs of M. incognita per plant, when incubated for 28 days at 32.5 C.

From the results obtained in the screening work, it appears that some progress may be expected in the selection for resistance to M. incognita at high temperature, especially resistance to the less aggressive race 1. The best strategy for future screening work may be to inoculate the tomato seedlings with gradually increasing numbers of root-knot nematode eggs in the inoculum. This approach allows genetic fixation of resistance at the lower inoculum concentrations incubated at high temperature. As the inoculum is increased in subsequent tests it may be possible to detect superior genetic recombinations after intercrossing the previous selections.

Field Experiments

The average gall index was observed over 3 tomato growing seasons on 6 entries planted at the AREC at Bradenton (Table 17). During the spring of 1978, the rate of infection was low, and the resistance in cultivars and hybrids possessing the Mi gene was effective. The small diameter of individual galls observed on susceptible cultivars suggested a low initial nematode population in the field. The results also indicated a non-uniform field infestation, which commonly occurs in the distribution of root-knot nematodes in natural field conditions (55). The soil temperature registered at the weather station was lower at the beginning (14-31 C) and higher at the end of the season (23-32); however, no actual soil temperature data were taken from the plots.

During the fall of 1978 all 6 tomato entries had gall indices higher than 4.0. The soil temperature registered at the weather

Table 17. Average gall index observed on 6 cultivars and F_1 hybrids during 3 growing seasons at the AREC, at Bradenton, Florida.

Cultivar or F_1 Hybrid	Gall index		
	Spring 78	Fall 78	Spring 79
Walter PF	3.7	4.9	5.0
Floradade	4.1	5.0	5.0
Walter PF x Patriot	0.4	4.2	5.0
Walter PF x 74T2	0.3	4.6	5.0
Patriot	0.0	4.7	5.0
74T2	0.3	4.9	5.0

[a]Gall index: 0--0 gall per plant, 1--1 to 2, 2--3 to 10, 3--11 to 30, 4--31 to 100, and 5--more than 100 galls per plant.

station indicated higher temperatures during the early growing season (22-32 C). A higher initial nematode population in the field was indicated by the presence of large galls in susceptible cultivars. The occurrence of higher temperatures may also have contributed to more pronounced galling on cultivars and hybrids carrying the Mi gene.

In the spring of 1979 field experiment, artificial inoculations of 10,000 eggs of M. incognita race 4 were used. The soil temperature early in the season was lower than that in May/June. The galling produced in all cultivars and F_1 hybrids was the heaviest observed in all 3 seasons, and it was uniformly distributed in the field. Individual galls were not observed in most tomato entries, but the galling was presented as a continuous enlargement extending over the entire root system. In the cultivar 74T2, however, there was a higher number of individual galls, which may indicate that some level of resistance was operating despite the maximum rating for gall index (Table 17).

The inoculated tomato plants in the spring of 1979 test were shorter than the non-inoculated plants, indicating that the critical nematode density (68) had been exceeded. At the end of the experiment it was observed that an infestation of Xanthomonas vesicatoria was more severe on plants infected with root-knot nematode, indicating a predisposition to bacterial infection in plants weakened by nematodes. These results agree with those presented by Mjuge and Estey (57) demonstrating that the process of aging is accelerated by infection with M. incognita and that the root-knot nematode provokes profound physiological changes not only in the roots but in the entire plant. The results also agree with Wallace (85) who reported that under a high inoculum level there was a marked decrease in photosynthesis, and its influence on reduction

of top growth was due mainly to inhibition of upward translocation of water and nutrients.

The effects of the nematode infestation on tomato yield, number of fruits, and average weight of fruits are shown in Table 18. All three factors were reduced by M. incognita infection. The yield reduction (Table 18) varied from 15.98% in the cultivar 74T2 to 22.82% in the F_1 of Walter PF x Floradade. There was a tendency in cultivars carrying the Mi gene, Patriot and 74T2, to have a lower yield reduction than cultivars without the Mi gene or F_1 hybrids with the Mi//Mi^+ genotype. The higher yields were produced by the cultivar Floradade and F_1 Walter PF x 74T2. The biggest fruit size was observed in the cultivar 74T2.

Table 18. Yield, yield reduction, number of fruits, and average fruit weight observed in 6 tomato cultivars or hybrids on field non-inoculated or inoculated with 10,000 eggs of M. incognita-race 4 per plant in the spring 1979 at the AREC at Bradenton, Florida.

Cultivar or F_1 hybrid	Yield[a] tons/ha		Yield reduction[a] tons/ha	Fruits[a] 1,000/ha		Average fruit weight[a] grams	
	Inoc.(A)	Non-inoc.(B)	B-A	Inoc.	Non-inoc.	Inoc.	Non-inoc.
Walter PF	68.4 b	86.0 b	17.6 a	674.5 bc	799.5 bc	102 cd	108 cd
Floradade	78.3 a	101.3 a	23.0 a	727.9 ab	863.7 ab	108 cd	117 cd
WPF x Patr.	66.8 b	86.5 b	19.7 a	800.9 a	893.5 a	84 e	97 de
WPF x 74T2	76.4 a	96.7 a	19.7 a	627.2 c	750.9 c	123 b	128 b
Patriot	60.6 c	70.0 c	13.4 a	755.6 ab	870.5 ab	81 e	82 e
74T2	54.2 c	64.5 c	10.3 a	391.3 d	413.0 d	140 a	157 a
Averages[b]	67.7 b	84.7 a	17.3	662.9 b	765.2 a	106 b	115 a

[a]Means followed by the same letter in the same column are not significantly different by Duncan's multiple range test (alpha = 0.05).

[b]Three separate tests of mean differences are made here, comparing inoculated vs. non-inoculated means for yield, number of fruits, and average fruit weight. Pairs of means followed by the same letter are not significantly different by Duncan's multiple range test (alpha = 0.05).

CONCLUSIONS

The best inoculum level for studies of resistance of Lycopersicon spp. to M. incognita and M. javanica is 200 eggs per plant. At this level individual egg masses can be counted and resistance to root-knot nematodes can be assessed quantitatively.

Thermoperiodism is an important phenomenon in maintaining resistance to M. incognita in tomato. Higher resistance in both L. peruvianum selections and L. esculentum cultivars were observed under diurnal soil temperature change (32.5 C for 12 hours-day/20-25 C for 12 hours-night) than at a continuous temperature of 32.5 C.

Race 4 of M. incognita found at the AREC, Bradenton, Florida, is more aggressive than race 1 of the same species collected in Gilchrist County, Florida. Race 4 of M. incognita is more aggressive toward the lines of L. esculentum studied than to the selections made from L. peruvianum var. dentatum accessions.

The critical testing period for resistance to M. incognita in controlled temperature tanks at 32.5 C seems to be the first 12 days after inoculation. Extending the experiment beyond 12 days at 32.5 C had little effect on nematode reproduction.

Some progress has been achieved in the search for improved resistance to M. incognita in selections of L. peruvianum var. dentatum and L. esculentum at 32.5 C, using an inoculum concentration of 200 eggs per plant. While plants of L. esculentum lines with low nematode

reproduction were obtained, a few plants of some selections of L. peruvianum var. dentatum showed no egg masses after several screening tests.

The selection 11-5-16 of L. peruvianum var. dentatum PI 129149 was the most resistant to M. incognita-race 1 in the entire screening work.

The yield, number of fruits, average fruit weight, and plant size were seriously affected among 4 cultivars and 2 F_1 hybrids inoculated with 10,000 eggs per plant of M. incognita-race 4 in a field experiment at the AREC, Bradenton, Florida, during the spring of 1979.

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APPENDICES

APPENDIX A

TOMATO ACCESSIONS CARRYING THE *Mi* GENE

Table A-1. *L. esculentum* cultivars and PI carrying the *Mi* gene mentioned in this dissertation.

Cultivar or PI No.	Cultivar or PI No.
Anahu	Nemared
Atkinson	Nematex
Auburn FMN76	Patriot
Hawaii 7153	Piersol
Healani	Rossol
Kewalo	74T2
Marsol	341128 (Nemared)
Monita	

APPENDIX B

PEDIGREES OF THE PI 129149 REGISTERED AT THE AREC, BRADENTON, FLORIDA

Table B-1. PI 129149 selections mentioned in this dissertation and correspondent pedigrees registered at the AREC, Bradenton, Florida.

PI selection	Correspondent pedigree at AREC
129149-11-5	129149-2(sib)-5
129149-12-1	129149-3(sib)-1
129149-SN6-R3-5	129149-2(sib)-2-5
129149-SN9-R4-1	129149-2(sib)-5-1
129149-SN9-R4-2	129149-2(sib)-5-2
129149-SN9-R4-4	129149-2(sib)-5-4
129149-SN11-R2-1	129149-3-1-1
129149-SN11-R2-2	129149-3-1-2
129149-SN11-R2-5	129149-3-1-5
129149-SN12-R1-4	129149-3-2-4(R1)
129149-SN12-R1-5	129149-3-2-5(R1)
129149-SN12-R1-6	129149-3-2-6(R1)
129149-SN12-R1-7	129149-3-2-7(R1)
129149-SN12-R2-1	129149-3-2-1(R2)
129149-SN12-R2-2	129149-3-2-2(R2)
129149-SN12-R2-5	129149-3-2-5(R2)
129149-SN12-R2-6	129149-3-2-6(R2)
129149-SN12-R2-7	129149-3-2-7(R2)
129149-SN13-R2-9	129149-3-3-9

BIOGRAPHICAL SKETCH

Marcelo de Targa Araujo has studied in agricultural schools since the age of twelve. At the age of eighteen he received a diploma for horticultural technician. In 1964 he graduated from the Federal University of Viçosa as an "Engineer" in agronomy.

He worked as a researcher with the Federal University of Viçosa on vegetable crops from January, 1965, to April, 1968. Simultaneously he took graduate courses and received the M.S. degree in phytotechny with special concentration in the area of vegetable crops. From May, 1968, to April, 1975, he worked as a researcher in vegetable crops with the Secretary of Agriculture in Brasilia, DF. From 1971 to 1975 he received a grant to work as an assistant researcher with the National Council for Research (Brazil). In this capacity he dedicated special attention to the introduction and evaluation of new cultivar of vegetable crops for the Federal District. The Federal District is located in the cerrado area, a region which constitutes 20 to 25% of the area of Brazil.

At present he is working on plant breeding projects with EMBRAPA, the Brazilian Agricultural Research System. He belongs to the Brazilian Olericulture Society in which he served as vice-president from 1971 to 1977. He also worked as a visiting professor at the University of Brasilia where he taught courses in vegetable crops which extended over two semesters.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mark J. Bassett

Dr. Mark J. Bassett, Chairman
Associate Professor of Vegetable Crops

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jimmy J. Augustine

Dr. Jimmy J. Augustine
Assistant Professor of Vegetable Crops

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

D. W. Dickson

Dr. Donald W. Dickson
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Buford D. Thompson
Professor of Vegetable Crops

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1979

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